TRADE SECRET

Study Title

H-28548: 21-Day Chronic, Static-Renewal Toxicity Test with the Cladoceran, *Daphnia magna*

TEST GUIDELINES: U.S. EPA Ecological Effects Test Guidelines
OPPTS 850.1300 (1996)

OECD Guideline for the Testing of Chemicals
Section 2 (Part 211) (1998)

AUTHOR: Robert A. Hoke, Ph.D.

STUDY COMPLETED ON: December 18, 2008

PERFORMING LABORATORY: E.I. du Pont de Nemours and Company
DuPont Haskell Global Centers
for Health & Environmental Sciences
P.O. Box 50
Newark, Delaware 19714
U.S.A.

LABORATORY PROJECT ID: DuPont-17751-254

WORK REQUEST NUMBER: 17751

SERVICE CODE NUMBER: 254

SPONSOR: E.I. du Pont de Nemours and Company
Wilmington, Delaware 19898
U.S.A.

GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

This study was conducted in compliance with U.S. EPA FIFRA (40 CFR part 160) Good Laboratory Practice Standards, which are compatible with current OECD and MAFF (Japan) Good Laboratory Practices.

Applicant/Sponsor: E.I. du Pont de Nemours and Company
Wilmington, Delaware 19898
U.S.A.

Study Director: Robert A. Hoke _____ 10 Dec 2008
Robert A. Hoke — Date
Principal Research Ecotoxicologist and Technical Group Leader

Applicant/Sponsor: _____ _____
DuPont Representative — Date

QUALITY ASSURANCE STATEMENT

Work Request Number: 17751
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Phase Audited	*Audit Dates*	*Date Reported to Study Director*	*Date Reported to Management*
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Report/Records:	December 04, 05, 09, 2008	December 09, 2008	December 15, 2008

Reported by: Donna M. Johnston 17 Dec. 2008
Donna M. Johnston
Quality Assurance Auditor
Date

CERTIFICATION

We, the undersigned, declare that this report provides an accurate evaluation of data obtained from this study.

In-Life Evaluation by: Barbra D. Ferrell — 16-Dec-2008
Barbra D. Ferrell, B.S.
Associate Scientist
Date

Analytical Evaluation by: Peter Bloxham — 17-DEC-2008
Peter Bloxham, Ph.D.
Senior Research Chemist
Date

Statistical Analysis by: John W. Green — 18-Dec-2008
John W. Green, Ph.D., Ph.D.
Principal Research Statistician
Date

Reviewed by: Alan Samel — 17 Dec 2008
Alan Samel, M.S.
Research Ecotoxicologist
Date

Issued by Study Director: Robert A. Hoke — 18 Dec 2008
Robert A. Hoke, Ph.D.
Principal Research Ecotoxicologist and Technical Group Leader
Date

TABLE OF CONTENTS

LIST OF TABLES

Page

LIST OF FIGURES

Page

LIST OF APPENDICES

Page

STUDY INFORMATION

Substance Tested:
- HFPO Dimer Acid Ammonium Salt
- 2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)propionic acid, ammonium salt
- 62037-80-3 (CAS Number)
- H-28548

Haskell Number: 28548

Composition:

84%	HFPO Dimer Acid Ammonium Salt
12.7%	Water
150 ppm	Perfluorooctanoic acid

Purity: 84% (based on Certificate of Analysis)

Physical Characteristics: Clear and colorless liquid

Stability: The test substance was stable under the conditions of the study.

Study Initiated/Completed: September 4, 2008 / (see report cover page)

Experimental Start/Termination: September 10, 2008 / October 9, 2008

SUMMARY

GUIDELINES: The effect of H-28548 (purity 84%) on the survival, growth, and reproduction of *Daphnia magna* was assessed in a chronic, unaerated 21-day static-renewal test in accordance with the appropriate Good Laboratory Practice standards and test guidelines (U.S. EPA OPPTS 850.1300; OECD 211).

NOMINAL TEST CONCENTRATIONS: 2.5, 5, 10, 20, and 40 mg/L H-28548, dilution water control

MEAN, MEASURED TEST CONCENTRATIONS: 2.13, 4.17, 8.13, 16.2, and 33.0 mg/L H-28548, none detected in dilution water control

AGE OF TEST ORGANISMS: Less than 24 hours old at test start

SOURCE OF TEST ORGANISMS: DuPont Haskell in-house culture

TEST DESIGN: A total of 10 replicates, each containing one, <24 hour-old neonate, was tested per concentration (10 neonates/concentration) and dilution water control. Samples of newly prepared H-28548 test solutions were taken for test concentration verification on days 0, 2, 9, 16, and 19 before test solutions were poured into appropriate test chambers. Samples from old H-28548 test solutions were taken for test concentration verification on days 5, 12, 19, and 21. Replicates used for sampling old test solutions were determined by random number prior to the start of the study. The same replicates were used for analytical sampling throughout the study unless there was adult immobility in a replicate. Alternative replicates for sampling were determined by random numbers prior to the start of the study. Test solutions were renewed every Monday, Wednesday, and Friday during the study. The daphnids were fed *Pseudokirchneriella subcapitata* at a rate of approximately 62,500 cells/mL of test solution and 2.1 mL/L of a yeast, cereal leaves and trout chow mixture (YCT) on a daily basis. The combination of alga and YCT was equivalent to approximately 0.1-0.2 mg total organic carbon (TOC) per daphnid. Test solutions were maintained between 19.3 and 21.0°C (mean 20.2°C). Observations of immobilization, sublethal effects, and production of young were made daily. Length and dry weight of surviving adult daphnids were determined at test end.

CONCLUSION: The 21-day NOEC (no-observed-effect concentration) for H-28548 using *Daphnia magna* neonates exposed for 21 days under static-renewal conditions was 4.17 mg/L, based on mean, measured test concentrations and the total number of live young produced per surviving female at the end of the study. The MATC (maximum-acceptable-toxicant concentration), and LOEC (lowest-observed-effect concentration) were 6.15 and 8.13 mg/L, respectively. The EC_{50} for adult survival was greater than 33.0 mg/L.

STUDY COMPLETION: (see report cover page)

INTRODUCTION

The purpose of this study was to assess the chronic toxicity of H-28548 to *Daphnia magna* based on survival, growth, and reproduction.

MATERIALS AND METHODS

A. Test Guidelines

The study design complied with the following test guidelines:

- U.S. EPA, OPPTS 850.1300: Daphnid Chronic Toxicity Test, *Ecological Effects Test Guidelines* (1998)
- OECD, Section 2 (Part 211): *Daphnia magna* Reproductive Test, *Guideline for the Testing of Chemicals* (1988)

B. Test Substance

The test substance, H-28548, was supplied by the sponsor as a clear and colorless liquid. The test substance consisted of 84% H-28548 by analysis (Appendix A).

The solubility of H-28072 (a different sample of the test substance) in Haskell Well Water (HWW) was verified at 218 mg/L at 10°C. The stability of H-28072 was demonstrated for up to 96 hours in a 11 mg/L solution in HWW at 4°C and 20°C.[(1)] Centrifugation of day 0 test solutions containing algae demonstrated that the presence of algae had no effect on the measured test substance concentrations (Table 2).

C. Test Solution Preparation

On day 0 and on each renewal day (Monday, Wednesday, and Friday), a primary stock solution of 40 mg/L was prepared by adding approximately 0.2 grams of H-28548 to 5000 mL of HWW in a 6-L Erlenmeyer flask and stirring for approximately 40 minutes. At test start, the stock solution was clear and colorless with no visible precipitate. Final nominal test concentrations of 2.5, 5, 10, 20, and 40 mg/L were prepared by diluting appropriate amounts of the primary stock solution with HWW, 2.1 mL/L of algae (final algal concentration of approximately 62,500 cells/mL in test solutions) and 2.1 mL/L of yeast, cereal leaves and trout chow mixture (YCT). Final test solutions were stirred for approximately 5 minutes before use. At test start, the test solutions were slightly cloudy and pale green in color (due to the presence of algae) with small particles of YCT visible.

D. Dilution Water

Dilution water originated from the Haskell well, which is 480-feet deep and is cased and sealed to bedrock. The hardness of the HWW is adjusted to approximately 100-140 mg/L as $CaCO_3$ by

the flow-proportioned addition of $CaCl_2$. The HWW is then aerated, passed through a green sand filter to remove iron, and filtered through 50-, 10-, and 3-μm filters to remove particulates. The water is heated or chilled as appropriate and distributed through aged polyvinyl chloride piping. The dilution water is analyzed at least once yearly for major anions and cations, metals, total organochlorine and organophosphate pesticides, and polychlorinated biphenyls (Table 1). The dilution water meets OECD and ASTM criteria and specifications.

E. Test Organism Culture

Daphnia magna were reared at DuPont Haskell in 1000-mL Pyrex® beakers (10 per beaker at culture initiation) which contained 1000 mL of aerated, filtered HWW held at approximately 20.0°C. Daphnids were fed on a daily basis with 3 mL/L of a yeast, cereal leaves and trout chow (YCT) mixture and the green alga, *Pseudokirchneriella subcapitata*, at a rate of approximately 62,500 cells/mL of culture media. The YCT mixture was standardized to 1700-2100 mg/L solids. The combination of YCT and alga is equivalent to approximately 0.1-0.2 mg total organic carbon per daphnid per day. YCT is analyzed periodically to assure that contaminants are not present at levels that would be expected to affect the scientific integrity of the study. Green algae are not routinely analyzed for contaminants because they are cultured in a defined laboratory medium prepared using reagent grade chemicals and deionized water. Neonates used in this test were less than 24 hours old and were collected from the 8th and 4th broods of 22- and 14-day old parent daphnids, respectively. Sickness, injury, and abnormalities were not seen and ephippia were not being produced by the parent daphnids. No adult immobility was seen during the 48-hour pre-test period in the culture used to supply neonates for testing. *Daphnia magna* were identified by labels on the culture beakers and test chambers.

F. Test Methods

A dilution water control and nominal concentrations of 2.5, 5, 10, 20, and 40 mg/L H-28548 were used in this study.

Pyrex® beakers (250 mL) containing 200 mL of test solution (6.5 cm depth) were used for testing. Each test concentration or dilution water control was replicated 10 times with one neonate per replicate. Daphnids were assigned to the test chambers using random numbers. Test concentrations were allocated to a constant-temperature, recirculating waterbath using random numbers and test chambers were covered with glass plates during the test. Daphnids were fed *Pseudokirchneriella subcapitata* and YCT at final concentrations of approximately 62,500 cells/mL and 2.1 mL/L of test solution, respectively, on a daily basis. The combination of algae and YCT was equivalent to approximately 0.1-0.2 mg TOC per daphnid. New test solutions were held between 19.7 and 20.8°C (mean 20.3°C) and old test solutions were held between 19.3 and 21.0°C (mean 20.0°C). Test solutions were not aerated during the study. Daphnids were transferred to new test solutions on renewal days using a glass pipette. A photoperiod of 16 hours light (approximately 143 - 293 Lux) and 8 hours darkness was employed, which included approximately 30 minutes of transitional light (approximately 6 - 41 Lux) to simulate dawn and dusk as part of the dark interval.

Observations were made daily on the number of surviving adult daphnids, occurrence of sublethal effects, and production of live or immobile young. The criterion for immobility was

the lack of response to application of a gentle stimulus. Immobile daphnids (adults and young) were discarded on renewal days. Live or immobile young were counted and removed on renewal days.

At the end of the test (21 days), the length of all surviving adult daphnids was measured to the nearest 0.1 mm by use of a calibrated ocular micrometer in the eyepiece of a dissecting microscope. The surviving adult daphnids were then weighed as follows:

- Surviving daphnids were pooled and placed in one weigh boat (identified by test concentration) for a maximum of 10 daphnids. Unique replicate identification was not maintained during this process.

- The daphnids were dried overnight (20-24 hours) at approximately 70°C in a drying oven.

- After drying, the weigh boats containing the daphnids were removed from the oven, allowed to cool in a dessicator for at least 1-2 hours, and then weighed. Each weigh boat containing the daphnids was weighed to the nearest 0.01 mg with a Mettler balance on the same day that drying was completed. Individual body weights were measured by placing the weigh boat containing the daphnids on the balance and recording the weight of the pan and the daphnids, removing one daphnid and recording the weight of the pan and the remaining daphnids with the difference being the weight of the individual daphnid, and repeating until all daphnids were weighed and the weigh boat was empty.

Dissolved oxygen concentration, pH, and temperature were measured in two randomly-selected replicates of the dilution water control and all test concentrations before daphnids were added at the beginning of the test, at test solution renewal (from both old and new solutions), and at test end. A continuous recording thermometer with probe placed in the waterbath was used to check for temperature variation during the study. Total alkalinity, EDTA hardness, and conductivity were measured in new solutions of the dilution water control and the highest test concentration with surviving test organisms at the beginning of the test, weekly thereafter, and in 1 replicate of old solutions at test end.

G. Sample Preparation and Chemical Analysis

1. Sample Collection and Treatment

One sample plus a back-up sample of each new test solution was received from each test concentration including the dilution water control on days 0, 2, 9,16, and 19 of the test before the test solutions were poured into the replicate test chambers. One sample plus a back-up sample of the old test solutions were received from 2 of the 10 replicate chambers (replicates D and F) from each test concentration including the dilution water control on days 5, 12, 19, and 21. Samples and back-up samples were stored refrigerated upon receipt and when not in use.

The day 0 samples from each test concentration including the dilution water control samples were analyzed with and without centrifugation to evaluate potential partitioning of the test substance to algae used as the daphnid diet. Aliquots of each of the samples were centrifuged for 30 minutes at a RCF setting of 20,817 ×g at 20°C before analysis. All other test samples were analyzed without centrifugation.

All test samples were prepared for analysis by adding 0.975 mL of Nanopure® water to an HPLC vial; 0.025 mL of each test sample was then added to the HPLC vial and the vial vortexed for 1 minute prior to sample analysis. This resulted in a 40× sample dilution factor.

Quality control (QC) samples were prepared by adding 0.965 mL of Nanopure® water for test solution QC samples or 0.975 mL of Nanopure® water for the control QC sample to 4 separate HPLC vials followed by addition of 0.025 mL of dilution water control to each of the 4 HPLC vials. Finally, 0.010 mL of a 2.00, 25.0, or 50.0 µg/mL analytical standard stock solution, dependent on level, was added to each HPLC vial containing a test solution QC sample and vortexed for 1 minute prior to analysis.

Concentrations of H-28548 were measured by high performance liquid chromatography with detection by mass spectrometry (LC/MS) within 3 days of sample submission. However, due to low recoveries for day 16 new and day 19 old and new samples, the back-up samples for these days were analyzed within 11 days of sample submission for confirmation of original results.

2. Instrument and Conditions

HPLC Instrument:	Agilent Model (formerly Hewlett Packard) 1100	
MS Instrument:	Waters Micromass ZQ	
Software:	Masslynx and Quanlynx software version 4.0	
LC Parameters:		
Column:	Zorbax SB-C8, 2.1 × 30 mm, 1.8 micron	
Mobile Phase:	A: 75% 0.15% Acetic acid in LC water B: 25% Acetonitrile	
Flow Rate:	0.400 mL/min	
Column Temperature:	35°C	
Injection Volume:	10 µL	
MS Parameters:		
Capillary Voltage:	3.2 kV	
Source Temperature:	120°C	
Desolvation Temperature:	300°C	
Cone Gas Flow:	50 L/Hr	
Desolvation Gas Flow:	400 L/Hr	
Quadrupole Resolution:	LM and HM = 15	
Ionization Mode:	Electrospray, Negative Ion	
Divert Valve:	NA	
Data Acquisition Function:	Mass	329
	Time Interval	0.00 to 4.00 minutes
Dwell	0.20 seconds	
Cone Voltage:	9.0 V	

3. Quantitation

A primary stock solution of the reference standard (i.e., the test substance, H-28548, purity 84%), was made by adding the standard to Nanopure® water. An aliquot of the primary stock was used to prepare a secondary stock and diluted with Nanopure® water. On each day of analysis an aliquot of the secondary stock was diluted with Nanopure® water to prepare a tertiary stock solution. Aliquots of the tertiary stock solution were diluted with Nanopure® water to prepare calibration standards with concentrations that bracketed the concentrations of the test solutions. Duplicate injections of test and calibration standard solutions were made and the peak areas were determined electronically.

The calibration standard curve was generated by regression analysis using the chromatographic peak areas of the calibration standard solutions. Data for test solutions were compared to the calibration standard curve to determine concentrations of H-28548. The limit of detection (LOD) was determined by calculating the average noise level in chromatograms of the dilution water control samples and comparing them to the signal of a calibration standard of known concentration. Two chromatograms were examined for noise-related peaks near the retention time of the analyte. The LOD was calculated as 3 times the concentration equivalent of the mean noise level. The limit of quantitation (LOQ) was established as the lowest calibration standard concentration.

H. Statistical Analyses

The NOEC, LOEC and MATC were calculated with respect to (1) the survival of adult *Daphnia magna*, (2) total number of live young per surviving female, (3) number of immobile young per surviving female, (4) the first day of reproduction, and (5) body length, and (6) dry weight of surviving adults at 21 days. Endpoint values were analyzed based on nominal test substance concentrations but are reported based on mean measured test concentrations.

The NOEC for each response was determined through the use of trend tests (Cochran-Armitage trend test or the Jonckheere trend test) wherever appropriate. These trend tests assume monotonicity in the concentration-response. Parametric or, if appropriate, non-parametric tests, were used to determine if there was significant departure from monotonicity. Other multiple comparison methods were used for non-monotone data.

Adult survival data (*i.e.*, number of live daphnids at 21 days) was evaluated by the Cochran-Armitage test [4] used in a step-down manner [5] with equally-spaced concentration scores. If significant departure from monotonicity occurred, then Fisher's exact test [4] was used with a Bonferonni correction. [5]

Continuous or reproduction data (*i.e.*, length, weight, immobile young, total live young produced, and first day of reproduction) were evaluated for normality using the Shapiro-Wilk test [6] while homogeneity of variance was assessed by Levene's test.[7] Data found to be both normal and homogenous were evaluated in the context of ANOVA.[5] Monotonic concentration-response data were evaluated using the Jonckheere -Terpstra trend test.[8] Non-monotonic concentration-response data were assessed using the Dunnett method [5] if the data were normally distributed and homogeneous, and using the Tamhane-Dunnett (or T3) method [5] if the data

were normally distributed but heterogeneous. If factors in addition to concentration were considered, such as the number of organisms in the test chamber at test start, then the Jonckheere test was not appropriate and multi-factor ANOVA was used for analysis. Non-normal data were analyzed by alternative methods.[7] If possible, a transformation was found to obtain normality. Otherwise, a Kruskal-Wallis test [8] replaced the ANOVA, Dunn's test [5] replaced Dunnett's, and the Jonckheere-Terpstra test was still applicable, except as noted. Outliers were determined by the Tukey outlier rule and, if present, their effect on the conclusions was determined.

If massive ties were present in a continuous response, exact [9] permutation data analysis methods were used for analysis. An exact version of the Kruskal-Wallis test was used for analysis, followed by exact versions of Jonckheere-Terpstra or separate Mann-Whitney [8] (or, equivalently, Wilcoxon) comparisons of each treatment group to the control, using a Bonferonni correction.

The No Observable Effect Concentration (NOEC) was defined as the highest measured concentration at or below which no statistically significant effect was observed. The Lowest Observed Effect Concentration (LOEC) was the lowest measured concentration at which a statistically significant effect occurred. The maximum acceptable toxicant concentration (MATC) was defined as the geometric mean of the NOEC and LOEC.

RESULTS AND DISCUSSION

A. Analytical Report

1. Chromatographic Results

H-28548 eluted as a well-resolved chromatographic peak with a retention time of approximately 2.1 to 2.3 minutes. A typical calibration standard curve is shown in Figure 1. Representative chromatograms of a calibration standard solution, a dilution water control solution sample, and a test solution sample are presented in Figures 2 to 4, respectively.

The LOD and LOQ were determined to be 0.04 mg/L and 0.200 mg/L, respectively, based on a 40× sample dilution factor.

2. QC Sample Results

The calculated recovery of the QC samples ranged from 94% to 104%. These data confirm accurate quantitation by the analytical method during the study.

3. Test Solution Results

Mean, measured concentrations of H-28548 ranged from 96 to 101% of the targeted nominal test concentrations adjusted for test substance purity of 84% (Table 2). All measured values of H-28548 were within 1.5× of the lowest value for all samples within a concentration. These data indicate that H-28548 concentrations were maintained at acceptable levels throughout the definitive test.

Control solutions contained no detectable concentrations of H-28548 (Table 2).

Centrifugation of test solutions on day 0 had no effect on the measured concentrations of H-28548 (Table 2).

B. In-Life Report

Nominal H-28548 concentrations selected for the study were 2.5, 5, 10, 20, and 40 mg/L. The corresponding mean, measured concentrations of H-28548 were 2.13, 4.17, 8.13, 16.2, and 33.0 mg/L. A dilution water control was used in this study. H-28548 was not detected in the dilution water control.

The most recent periodic analysis of dilution water (Table 1) and YCT used for culturing and testing *Daphnia magna* indicated that contaminants were not present at levels that would be expected to affect the scientific integrity of the study. Dilution water quality was acceptable based on OECD[2] and ASTM[3] dilution water criteria. All chemical and physical parameters for the 21-day study were within expected ranges (Tables 3-7). Total alkalinity, EDTA hardness, and conductivity values for the dilution water control throughout the study ranged from 86-87 mg/L as $CaCO_3$, 128-176 mg/L as $CaCO_3$, and 290-320 μmhos/cm, respectively. Total alkalinity, EDTA hardness, and conductivity values for the highest, mean measured test

concentration with surviving organisms (33.0 mg/L) throughout the study ranged from 88-90, 125-139, and 305-340, respectively. The overall temperature range for new and old test solutions, including the dilution water control, was between 19.3 and 21.0°C. The pH ranged from 7.8 to 8.2 in new solutions and from 7.5 to 8.1 in old solutions and did not deviate by more than 0.5 units over the 48- or 72-hour period between renewals within the same replicate at all concentration levels. The overall minimum dissolved oxygen concentration for the new and old test solutions, including the dilution water control, was 7.3 mg/L or 80.2% of saturation (based on an air saturation value of 9.1 mg/L at 20°C) throughout the study.

A summary of percent adult survival, first day of reproduction, total live young produced per surviving adult, total immobile young produced per surviving adult, and length and dry weight of surviving adults is presented in Table 8. Adult survival data are provided in Table 9, and individual reproduction, length, and weight data in Tables 10-14. Percent survival of adult daphnids in the dilution water control was 100% at the end of the study. Adult daphnids of the dilution water control produced an average of 143.7 total live young per surviving adult at the end of 21 days. No ephippia were seen at any test substance concentration or in the dilution water control.

C. Statistical Analyses

Immobility of adult *Daphnia magna* was analyzed for day 21 of the test. On day 21 there was at least 90% mobility in each test concentration group. The Fisher Exact test and the Cochran-Armitage trend test determined that the NOEC for survival of adult *Daphnia magna* on day 21 was >33 mg/L.

The total number of live young was analyzed on day 21 of the test. The data representing the number of live young on day 21 contained a large number of ties. The NOEC for the number of live young on day 21 was 4.17 mg/L using the Jonckheere-Terpstra test.

There were massive ties in the data reporting the number of immobile young per surviving adult on day 21 and the first day of reproduction. The NOECs for number of immobile young on day 21 was 8.13 mg/L while the NOEC for the first day of reproduction was >33.0 mg/L. The NOECs for the number of immobile young and first day of reproduction were determined using the Jonckheere-Terpstra test.

A standard one-way ANOVA was done on the data reporting the length of surviving adult *Daphnia magna*. The data were found to be normally distributed by the Shapiro-Wilk test. The Jonckheere-Terpstra test was used to determine that the NOEC for the length of surviving *Daphnia magna* at test end was >33.0 mg/L.

A standard one-way ANOVA was done on the data reporting the dry weight of surviving adult *Daphnia magna*. The data were found to be non-normally distributed by the Shapiro-Wilk test. The Jonckheere-Terpstra test was used to determine the NOEC for the dry weight of surviving *Daphnia magna* at test end was >33.0 mg/L.

CONCLUSION

The NOEC (no-observed-effect concentration), LOEC (lowest-observed-effect concentration), and MATC (maximum-acceptable-toxicant concentration) for H-28548 based on mean, measured test concentrations and the total number of live young produced per surviving female were 4.17 mg/L, 6.15 mg/L, and 8.13 mg/L for *Daphnia magna* exposed to H-28548 for 21 days. The EC_{50} for adult survival was greater than >33.0 mg/L.

RECORDS AND SAMPLE STORAGE

Specimens (if applicable), raw data, the protocol, amendments (if any), and the final report will be retained at DuPont Haskell, Newark, Delaware, Iron Mountain Records Management, Wilmington, Delaware, or Quality Associates Incorporated, Fulton, Maryland.

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TABLES

Table 1
Chemical Characteristics of Haskell Laboratory Well Water[a]

Parameter	MDL[b]	Analytical Value	Parameter	MDL[b]	Analytical Value
BOD, mg/L	2.8	ND[c]	Lead, mg/L	0.0069	ND
COD, mg/L	2.6	ND	Magnesium, mg/L	0.0135	6.02
DOC, mg/L	0.50	ND	Manganese, mg/L	0.00084	0.0020 J
TOC, mg/L	0.50	ND	MBAS/LAS, mg/L	0.035	ND
Total Kjeldahl N, mg/L	0.50	ND	Mercury, mg/L	0.000056	ND
Ammonia N, mg/L	0.03	ND	Nickel, mg/L	0.0056	ND
Turbidity, NTU	0.09	0.093 J[e]	Nitrite, mg/L	0.015	ND
Phenolics, mg/L	0.015	ND	Nitrate, mg/L	0.04	ND
Color, apparent Co/Pt[d]	5.0	ND	Ortho-phosphate, mg/L	0.01	0.015 J
Solids			Potassium, mg/L	0.0503	3.89
total suspended, mg/L	1.5	ND	Selenium, mg/L	0.0003	0.00094 J
Aluminum, mg/L	0.0802	ND	Silver, mg/L	0.000037	ND
Antimony, mg/L	0.0097	ND	Sodium, mg/L	0.433	8.49
Arsenic, mg/L	0.00095	ND	Sulfate, mg/L	0.3	3.0
Beryllium, mg/L	0.0009	ND	Sulfide, mg/L	0.054	ND
Boron, mg/L	0.0094	0.0182 J	Zinc, mg/L	0.0081	0.0104 J
Bromide, mg/L	0.4	ND	Ca/Mg	NA[f]	7.43
Cadmium, mg/L	0.00021	ND	Na/K	NA	2.18
Calcium, mg/L	0.0702	44.7	Volatile priority		
Chloride, mg/L	2.0	45.3	pollutants, µg/L	0.5-40	ND – 2 J
Chlorine, residual, mg/L	0.04	ND	Acid extractable		
Chromium, mg/L	0.00068	ND	priority pollutants, µg/L	1-19	ND
Cobalt, mg/L	0.0021	ND	Base/neutral		
Copper, mg/L	0.00038	0.0045	priority pollutants, µg/L	1-19	ND
Cyanide, mg/L	0.005	ND	Pesticides/PCBs, µg/L	0.0019-0.96	ND – 0.0066 J
Iron, mg/L	0.0522	ND	Organophosphate		
Fluoride, mg/L	0.08	0.31	pesticides, µg/L	0.48-1.4	ND

[a] Sample analyses performed at Lancaster Laboratories, Lancaster, Pennsylvania, date of sample collection 07 August 2008 unless indicated otherwise,
[b] MDL = method detection limit, [c]ND indicates not detected at the MDL, [d]Units based on cobalt/platinum reference, [e]A "J" follows analytical values which were greater than the MDL but less than the limit of quantitation, [f]NA = not applicable.

Table 2
Measured Concentrations of H-28548 in Test Solutions

Nominal H-28548 Concentration (mg/L)	Corrected Nominal H-28548 Concentration (mg/L)[a]	Measured H-28548 Concentration (mg/L)									Mean, Measured Concentration (mg/L)[c]	Percent Recovery (%)[d]
		Day 0 (New)[b]	Day 2 (New)[b]	Day 5 (Old)	Day 9 (New)[b]	Day 12 (Old)	Day 16 (New)[b]	Day 19 (Old)	Day 19 (New)[b]	Day 21 (Old)		
Water Control D	0.0	ND[e]	ND	ND	ND	ND	ND	ND	ND	ND	ND	---
Water Control F	0.0			ND		ND		ND		ND		
2.5 D	2.10	2.29	1.98	2.25	2.36	2.12	2.10	2.16	1.92	2.14	2.13	101
2.5 F	2.10	2.13[f]		2.22		2.03		2.03		2.07		
			average	2.24		2.08		2.10		2.11		
5.0 D	4.20	4.18	3.94	4.69	4.39	4.08	4.27	4.32	3.99	4.00	4.17	99
5.0 F	4.20	4.12[f]		4.36		4.12		3.91		4.08		
			average	4.53		4.10		4.11		4.04		
10.0 D	8.40	8.42	7.80	8.11	8.36	7.85	8.32	8.08	8.01	8.33	8.13	97
10.0 F	8.40	8.15[f]		8.08		8.10		7.83		8.11		
			average	8.10		7.98		7.96		8.22		
20.0 D	16.8	16.4	15.6	16.2	16.8	15.6	16.5	16.1	16.0	16.7	16.2	96
20.0 F	16.8	16.2[f]		16.9		15.7		15.0		16.0		
			average	16.6		15.7		15.6		16.4		
40.0 D	33.6	32.3	31.9	33.3	35.9	30.9	34.4	31.6	33.6	32.1	33.0	98
40.0 F	33.6	31.2[f]		34.9		31.3		29.3		33.9		
			average	34.1		31.1		30.5		33.0		

a Nominal H-28548 concentrations corrected for 84% purity by analysis.
b One sample of freshly prepared test solution was taken from each concentration level on days 0, 2, 9, 16, and 19 before the test solutions were poured into the replicate test chambers.
c Mean, measured H-28548 concentration was calculated as:
(Day 0 + Day 2 + Avg. Day 5 + Day 9 + Avg. Day 12 + Day 16 + Avg. Day 19 Old + Day 19 New + Avg. Day 21) / number of sample intervals
d Based on nominal concentration adjusted for purity.
e ND denotes less than limit of detection. The limit of detection for H-28548 was 0.04 mg/L based on a 40x sample dilution factor.
f Day 0 samples were also centrifuged to verify that there was no significant effect on the measured concentration due to binding of test substance to the daphnid diet. The values were not included in the calculation of the mean, measured concentrations of H-28548.

Table 3
Water Chemistry of the Dilution Water Control and Highest Mean Measured Test Substance Concentration with Surviving Organisms

Day	Total Alkalinity (mg/L as $CaCO_3$)	EDTA Hardness (mg/L as $CaCO_3$)	Conductivity (µmhos/cm)
Dilution Water Control			
0	87	176	320
7	87	130	300
14	86	129	310
21 (Rep E)	87	128	290
33.0 mg/L			
0	89	139	340
7	90	128	320
14	88	134	330
21 (Rep E)	90	125	305

Table 4
Temperature of the Waterbath

Day	Waterbath Temperature (°C)
0	20.3
1	20.3
2	20.1
3	20.1
4	19.9
5	20.1
6	20.3
7	20.3
8	20.1
9	20.5
10	20.8
11	20.5
12	20.5
13	19.4
14	19.5
15	19.2
16	19.6
17	20.2
18	20.1
19	19.4
20	19.2
21	19.4

Table 5
Temperature of H-28548 Test Solutions

(New Solutions)

		Dilution	Temperature (°C)				
		Water	Mean, Measured Concentrations (mg/L)				
Day	Replicate	Control	2.13	4.17	8.13	16.2	33.0
0	A	20.4	20.4	20.5	20.4	20.5	20.5
0	I	20.5	20.4	20.5	20.5	20.5	20.5
2	A	20.0	20.1	20.1	20.1	20.1	20.1
2	I	20.2	20.2	20.2	20.3	20.2	20.2
5	A	20.3	20.3	20.3	20.3	20.3	20.3
5	I	20.4	20.4	20.4	20.4	20.4	20.4
7	A	20.3	20.3	20.3	20.3	20.3	20.3
7	I	20.4	20.4	20.4	20.4	20.4	20.4
9	A	20.6	20.7	20.7	20.7	20.7	20.7
9	I	20.7	20.7	20.8	20.8	20.7	20.8
12	A	20.5	20.5	20.6	20.6	20.6	20.6
12	I	20.6	20.6	20.6	20.7	20.7	20.7
14	A	19.7	19.8	19.8	19.8	19.8	19.8
14	I	19.9	20.0	19.9	20.0	19.9	20.0
16	A	19.8	19.9	19.7	19.9	19.8	19.9
16	I	19.9	20.0	20.0	20.1	20.0	20.1
19	A	20.0	20.1	20.1	20.2	20.1	20.2
19	I	20.1	20.2	20.1	20.2	20.2	20.2

Temperature (°C): New Solutions

Mean	20.3
Minimum	19.7
Maximum	20.8

Table 5
Temperature of H-28548 Test Solutions (Continued)

(Old Solutions)

Day	Replicate	Dilution Water Control	Temperature (°C) Mean, Measured Concentrations (mg/L) 2.13	4.17	8.13	16.2	33.0
2	A	19.9	19.9	19.9	19.9	19.9	19.9
2	I	20.0	20.0	20.0	20.0	20.0	20.0
5	A	20.0	20.1	20.1	20.2	20.1	20.2
5	I	20.1	20.2	20.1	20.2	20.1	20.2
7	A	20.4	20.4	20.4	20.3	20.3	20.3
7	I	20.4	20.4	20.4	20.5	20.5	20.5
9	A	20.5	20.5	20.5	20.5	20.5	20.5
9	I	20.6	20.6	20.6	20.6	20.6	20.6
12	A	20.7	20.7	20.8	20.7	20.8	20.8
12	I	20.8	20.8	21.0	20.8	20.9	20.9
14	A	19.5	19.6	19.6	19.5	19.6	19.5
14	I	19.7	19.7	19.6	19.7	19.6	19.6
16	A	19.6	19.6	19.6	19.6	19.6	19.6
16	I	19.6	19.7	19.6	19.6	19.6	19.6
19	A	19.7	19.9	19.8	19.8	19.8	19.8
19	I	19.9	19.9	19.9	19.9	19.8	19.8
21	A	19.4	19.4	19.5	19.4	19.4	19.4
21	I	19.3	19.5	19.4	19.4	19.4	19.3

Temperature (°C): Old Solutions

Mean	20.0
Minimum	19.3
Maximum	21.0

Table 6
pH of H-28548 Test Solutions

(New Solutions)

		Dilution Water	pH Mean, Measured Concentrations (mg/L)				
Day	Replicate	Control	2.13	4.17	8.13	16.2	33.0
0	A	7.9	8.0	8.1	8.1	8.1	8.1
0	I	8.0	8.0	8.1	8.1	8.1	8.1
2	A	8.0	8.1	8.1	8.1	8.2	8.2
2	I	8.0	8.1	8.1	8.1	8.2	8.2
5	A	7.8	7.9	8.0	8.1	8.1	8.2
5	I	7.8	8.0	8.1	8.1	8.1	8.2
7	A	7.8	8.0	8.0	8.1	8.1	8.1
7	I	7.9	8.0	8.0	8.1	8.1	8.1
9	A	7.9	8.0	8.0	8.1	8.1	8.1
9	I	7.9	8.0	8.0	8.1	8.1	8.1
12	A	7.9	8.0	8.0	8.1	8.1	8.1
12	I	7.9	8.0	8.0	8.1	8.1	8.1
14	A	8.1	8.1	8.1	8.1	8.2	8.2
14	I	8.1	8.1	8.1	8.1	8.2	8.2
16	A	8.0	8.0	8.0	8.1	8.1	8.1
16	I	8.0	8.0	8.1	8.1	8.1	8.1
19	A	7.9	8.0	8.1	8.1	8.1	8.1
19	I	7.9	8.0	8.1	8.1	8.1	8.1

pH: New Solutions	
Minimum	7.8
Maximum	8.2

Table 6
pH of H-28548 Test Solutions (Continued)

(Old Solutions)

		Dilution Water	pH Mean, Measured Concentrations (mg/L)				
Day	Replicate	Control	2.13	4.17	8.13	16.2	33.0
2	A	8.0	8.1	8.1	8.1	8.1	8.1
2	I	8.1	8.1	8.1	8.1	8.1	8.1
5	A	7.9	8.0	8.0	8.1	8.1	8.1
5	I	8.0	8.0	8.1	8.1	8.1	8.1
7	A	7.9	8.0	8.0	8.0	8.0	8.0
7	I	8.0	8.0	8.0	8.0	8.0	8.0
9	A	7.9	8.0	8.0	8.1	8.1	8.1
9	I	8.0	8.0	8.1	8.1	8.1	8.1
12	A	7.7	7.8	7.9	7.9	7.9	7.9
12	I	7.8	7.9	7.9	7.9	7.9	7.9
14	A	7.9	8.0	8.0	8.0	8.0	8.0
14	I	7.9	8.0	7.9	8.0	8.0	8.0
16	A	7.9	7.9	7.9	8.0	8.0	8.0
16	I	7.9	8.0	8.0	8.0	8.0	8.0
19	A	7.7	7.9	7.9	8.0	8.0	8.0
19	I	7.8	7.9	7.9	7.9	7.9	8.0
21	A	7.5	7.7	7.8	7.8	7.9	7.8
21	I	7.6	7.7	7.8	7.8	7.9	7.9

pH: Old Solutions

Minimum	7.5
Maximum	8.1

Table 7
Dissolved Oxygen Concentration of H-28548 Test Solutions

(New Solutions)

		Dilution Water	Dissolved Oxygen Concentration (mg/L)[a] Mean, Measured Concentrations (mg/L)				
Day	Replicate	Control	2.13	4.17	8.13	16.2	33.0
0	A	8.4	8.5	8.4	8.4	8.3	8.4
0	I	8.5	8.4	8.4	8.4	8.3	8.4
2	A	8.3	8.3	8.3	8.1	8.1	8.0
2	I	8.3	8.3	8.2	8.1	8.1	8.0
5	A	8.2	8.2	8.2	8.2	8.2	8.2
5	I	8.2	8.2	8.2	8.2	8.2	8.2
7	A	8.2	8.1	8.1	8.0	8.0	7.9
7	I	8.1	8.1	8.1	8.0	8.0	7.9
9	A	8.2	8.1	8.1	8.1	8.1	8.0
9	I	8.2	8.1	8.1	8.1	8.1	8.0
12	A	8.0	8.0	8.1	8.0	8.0	8.0
12	I	8.0	8.1	8.0	8.0	8.0	8.0
14	A	8.4	8.4	8.4	8.3	8.4	8.2
14	I	8.5	8.4	8.4	8.4	8.3	8.2
16	A	8.3	8.3	8.3	8.3	8.3	8.3
16	I	8.2	8.4	8.4	8.3	8.3	8.4
19	A	8.3	8.3	8.3	8.2	8.2	8.1
19	I	8.3	8.3	8.3	8.2	8.2	8.1
Dissolved Oxygen Concentration (mg/L): New Solutions							
						Mean	8.2
						Minimum	7.9
						Maximum	8.5

a The theoretical dissolved oxygen concentration at saturation at 20°C = 9.1 mg/L.

Table 7
Dissolved Oxygen Concentration of H-28548 Test Solutions (Continued)

(Old Solutions)

Day	Replicate	Dilution Water Control	Dissolved Oxygen Concentration (mg/L)[a] Mean, Measured Concentrations (mg/L) 2.13	4.17	8.13	16.2	33.0
2	A	8.5	8.6	8.6	8.6	8.5	8.5
2	I	8.7	8.6	8.7	8.5	8.7	8.6
5	A	8.5	8.6	8.3	8.4	8.5	8.3
5	I	8.4	8.5	8.4	8.7	8.6	8.5
7	A	8.3	8.3	8.4	8.4	8.1	8.2
7	I	8.3	8.4	8.4	8.1	8.2	8.2
9	A	8.0	8.2	8.1	8.3	8.0	8.1
9	I	8.0	8.4	8.3	8.1	8.0	7.8
12	A	7.7	7.8	7.5	7.8	7.8	7.7
12	I	7.7	7.9	7.6	7.4	7.7	7.5
14	A	7.9	8.0	7.7	7.7	7.6	7.7
14	I	7.7	7.6	7.6	7.5	7.6	7.3
16	A	8.2	8.1	7.8	8.0	7.8	7.7
16	I	7.9	8.0	8.0	7.7	7.8	7.7
19	A	7.7	8.1	8.0	8.0	7.9	7.9
19	I	7.9	7.7	7.4	7.5	7.6	7.4
21	A	8.0	8.2	8.1	7.9	8.0	7.9
21	I	7.8	8.0	7.7	7.6	7.8	7.7

Dissolved Oxygen Concentration (mg/L): Old Solutions

Mean	8.0
Minimum	7.3
Maximum	8.7

a The theoretical dissolved oxygen concentration at saturation at 20°C = 9.1 mg/L.

Table 8
Summary of Data for *Daphnia magna* Exposed to H-28548 Test Solutions for 21 Days

Mean, Measured Concentrations (mg/L)	Percent Adult Survival[a]	Mean (Standard Deviation)				
		First Reproduction Day[b]	Total Live Young[c]	Total Immobile Young[d]	Adult Length (mm)[e]	Adult Dry Weight (mg)[e]
Dilution Water Control[f]	100	9.1 (0.3)	143.7 (16.2)	0 (0)	4.32 (0.19)	0.90 (0.13)
2.13	100	9.5 (0.7)	132.4 (21.0)	2.3 (6.3)	4.50 (0.24)	0.81 (0.22)
4.17	100	9.2 (0.6)	137.1 (21.7)	8.5 (16.3)	4.42 (0.31)	0.77 (0.18)
8.13	90	9.0 (0.0)	126.7 (14.3)	2.6 (4.6)	4.28 (0.20)	0.77 (0.12)
16.2	100	9.2 (0.4)	124.1 (19.1)	11.9 (14.8)	4.42 (0.19)	0.82 (0.11)
33.0	100	9.6 (1.0)	122.5 (21.2)	7.8 (10.9)	4.38 (0.20)	0.79 (0.24)

a Percent of adult daphnids alive at the end of the test
b First day that reproduction was observed
c Sum of live young produced per surviving adult in 21 days
d Sum of immobile young produced per surviving adult in 21 days
e Per surviving adult
f Haskell well water

Table 9
Immobility of Adult *Daphnia magna* Exposed to H-28548 for 21 Days

	Number Surviving									
	Replicate									
Day	A†	B†	C†	D†	E†	F†	G†	H†	I†	J†
Dilution Water Control										
1	1	1[d]	1	1	1	1	1	1	1	1
2	1	1	1	1	1	1	1	1	1	1
3	1	1	1[d]	1	1	1	1	1	1	1
4	1	1	1	1	1	1	1	1	1	1
5	1	1	1	1	1	1	1	1	1	1
6	1	1	1	1	1	1	1	1	1	1
7	1	1	1	1	1	1	1	1	1	1
8	1	1	1	1	1	1	1	1	1	1
9	1	1	1	1	1	1	1	1	1	1
10	1	1	1	1	1	1	1	1	1	1
11	1	1	1	1	1	1	1	1	1	1
12	1	1	1	1	1	1	1	1	1	1
13	1	1	1	1	1	1	1	1	1	1
14	1	1	1	1	1	1	1	1	1	1
15	1	1	1	1	1	1	1	1	1	1
16	1	1	1	1	1	1	1	1	1	1
17	1	1	1	1	1	1	1	1	1	1
18	1	1	1	1	1	1	1	1	1	1
19	1	1	1	1	1	1	1	1	1	1
20	1	1	1	1	1	1	1	1	1	1
21	1	1	1	1	1	1	1	1	1	1

† One daphnid per replicate chamber at test start.

[a] lethargic
[b] visibly small in size
[c] pale in color
[d] floating at surface
[e] accidentally crushed by pipette during transfer
[f] male
[g] aborted eggs
[h] ephippia

Table 9
Immobility of Adult *Daphnia magna* Exposed to H-28548 for 21 Days (Continued)

	Number Surviving									
	Replicate									
Day	A†	B†	C†	D†	E†	F†	G†	H†	I†	J†
2.13 mg/L										
1	1	1	1	1	1	1	1	1	1	1
2	1	1	1	1	1	1	1	1	1[b]	1
3	1	1	1[b]	1	1	1	1	1	1[b,c]	1
4	1	1	1	1	1	1	1	1	1[b,c]	1
5	1	1	1	1	1	1	1	1[d]	1[b,c]	1
6	1	1	1	1	1	1	1	1	1[b,c]	1
7	1	1	1	1	1	1	1	1	1[b]	1
8	1	1	1	1	1	1	1	1	1[b]	1
9	1	1	1	1	1	1	1	1	1	1
10	1	1	1	1	1	1	1	1	1	1
11	1	1	1	1	1	1	1	1	1	1
12	1	1	1	1	1	1	1	1	1	1
13	1	1	1	1	1	1	1	1	1	1
14	1	1	1	1	1	1	1	1	1	1
15	1	1	1	1	1	1	1	1	1	1
16	1	1	1	1	1	1	1	1	1	1
17	1	1	1	1	1	1	1	1	1	1
18	1	1	1	1	1	1	1	1	1	1
19	1	1	1	1	1	1	1	1	1	1
20	1	1	1	1	1	1	1	1	1	1
21	1	1	1	1	1	1	1	1	1	1

† One daphnid per replicate chamber at test start.

[a] lethargic
[b] visibly small in size
[c] pale in color
[d] floating at surface
[e] accidentally crushed by pipette during transfer
[f] male
[g] aborted eggs
[h] ephippia

Table 9
Immobility of Adult *Daphnia magna* Exposed to H-28548 for 21 Days (Continued)

	Number Surviving									
	Replicate									
Day	A†	B†	C†	D†	E†	F†	G†	H†	I†	J†
4.17 mg/L										
1	1	1	1	1	1	1	1	1	1	1
2	1	1	1	1	1	1	1	1	1	1
3	1[b]	1	1	1	1	1	1[d]	1	1[b]	1
4	1[b]	1	1	1	1	1	1	1	1[b]	1
5	1	1	1	1	1	1	1	1	1[b]	1
6	1	1	1	1	1	1	1	1	1[c]	1
7	1	1	1	1	1	1	1	1	1[b]	1
8	1	1	1	1	1	1	1	1	1	1
9	1	1	1	1	1	1	1	1	1	1
10	1	1	1	1	1	1	1	1	1	1
11	1	1	1	1	1	1	1	1	1	1
12	1	1	1	1	1	1	1	1	1	1
13	1	1	1	1	1	1	1	1	1	1
14	1	1	1	1	1	1	1	1	1	1
15	1	1	1	1	1	1	1	1	1	1
16	1	1	1	1	1	1	1	1	1	1
17	1	1	1	1	1	1	1	1	1	1
18	1	1	1	1	1	1	1	1	1	1
19	1	1	1	1	1	1	1	1	1	1
20	1	1	1	1	1	1	1	1	1	1
21	1	1	1	1	1	1	1	1	1	1

† One daphnid per replicate chamber at test start.

[a] lethargic
[b] visibly small in size
[c] pale in color
[d] floating at surface
[e] accidentally crushed by pipette during transfer
[f] male
[g] aborted eggs
[h] ephippia

Table 9
Immobility of Adult *Daphnia magna* Exposed to H-28548 for 21 Days (Continued)

	Number Surviving									
	Replicate									
Day	A†	B†	C†	D†	E†	F†	G†	H†	I†	J†
8.13 mg/L										
1	1	1	1	1	1	1	1	1	1	1
2	1	1	1	1	1	1	1	1	1	1
3	1	1	1	1	1	1	1	1	1	1
4	1	1	1	1	1	1	1	1	1	1
5	1	1	1	1	1	1	1	1	1	1
6	1	1	1	1	1	1	1	1	1	1
7	1	1	1	1	1	1	1	1	1	1
8	1	1	1	1	1	1	1	1	1	1
9	1	1	1	1	1	1	1	1	1	1
10	1	1	1	1	1	1	1	1	1	1
11	1	1	1	1	1	1	1	1	1	1
12	1	1	1	1	1	1	1	1	1	1
13	1	1	1	1	1	1	1	1	1	1
14	1	1	1	1	1	1	0	1	1	1
15	1	1	1	1	1	1	0	1	1	1
16	1	1	1	1	1	1	0	1	1	1
17	1	1	1	1	1	1	0	1	1	1
18	1	1	1	1	1	1	0	1	1	1
19	1	1	1	1	1	1	0	1	1	1
20	1	1	1	1	1	1	0	1	1	1
21	1	1	1	1	1	1	0	1	1	1

† One daphnid per replicate chamber at test start.

[a] lethargic
[b] visibly small in size
[c] pale in color
[d] floating at surface
[e] accidentally crushed by pipette during transfer
[f] male
[g] aborted eggs
[h] ephippia

Table 9
Immobility of Adult *Daphnia magna* Exposed to H-28548 for 21 Days (Continued)

	Number Surviving									
	Replicate									
Day	A†	B†	C†	D†	E†	F†	G†	H†	I†	J†
16.2 mg/L										
1	1	1	1	1	1	1	1	1	1	1
2	1	1	1	1	1	1	1	1	1	1
3	1[d]	1	1	1	1	1	1	1	1	1
4	1	1	1	1	1	1	1	1	1	1
5	1	1	1	1	1	1	1	1	1	1
6	1	1	1	1	1	1	1[d]	1	1	1
7	1	1	1	1	1	1	1	1	1	1
8	1	1	1	1	1	1	1	1	1	1
9	1	1	1	1	1	1	1	1	1	1
10	1	1	1	1	1	1	1	1	1	1
11	1	1	1	1	1	1	1	1	1	1
12	1	1	1	1	1	1	1	1	1	1
13	1	1	1	1	1	1	1	1	1	1
14	1	1	1	1	1	1	1	1	1	1
15	1	1	1	1	1	1	1	1	1	1
16	1	1	1	1	1	1	1	1	1	1
17	1	1	1	1	1	1	1	1	1	1
18	1	1	1	1	1	1	1	1	1	1
19	1	1	1	1	1	1	1	1	1	1
20	1	1	1	1	1	1	1	1	1	1
21	1	1	1	1	1	1	1	1	1	1

† One daphnid per replicate chamber at test start.

[a] lethargic
[b] visibly small in size
[c] pale in color
[d] floating at surface
[e] accidentally crushed by pipette during transfer
[f] male
[g] aborted eggs
[h] ephippia

Table 9
Immobility of Adult *Daphnia magna* Exposed to H-28548 for 21 Days (Continued)

	Number Surviving									
	Replicate									
Day	A†	B†	C†	D†	E†	F†	G†	H†	I†	J†
33.0 mg/L										
1	1	1	1	1	1[d]	1	1	1	1	1[d]
2	1	1	1	1	1[b]	1	1	1	1	1[b,c]
3	1	1	1	1	1[b,c]	1	1	1	1	1[b,c]
4	1	1	1	1	1[b,c]	1	1	1	1	1[b,c]
5	1	1	1	1	1[b,c]	1	1	1	1	1[b,c]
6	1	1	1	1	1[b,c]	1	1	1	1	1[b,c]
7	1	1	1	1	1[b]	1	1	1	1	1[b]
8	1	1	1	1	1[b]	1	1	1	1	1[b]
9	1	1	1	1	1	1	1	1	1	1
10	1	1	1	1	1	1	1	1	1	1
11	1	1	1	1	1	1	1	1	1	1
12	1	1	1	1	1	1	1	1	1	1
13	1	1	1	1	1	1	1	1	1	1
14	1	1	1	1	1	1	1	1	1	1
15	1	1	1	1	1	1	1	1	1	1
16	1	1	1	1	1	1	1	1	1	1
17	1	1	1	1	1	1	1	1	1	1
18	1	1	1	1	1	1	1	1	1	1
19	1	1	1	1	1	1	1	1	1	1
20	1	1	1	1	1	1	1	1	1	1
21	1	1	1	1	1	1	1	1	1	1

† One daphnid per replicate chamber at test start.

[a] lethargic
[b] visibly small in size
[c] pale in color
[d] floating at surface
[e] accidentally crushed by pipette during transfer
[f] male
[g] aborted eggs
[h] ephippia

Table 10
Number of Live Young Produced by *Daphnia magna* Exposed to H-28548 for 21 Days

	Number of Live Young Produced									
	Replicate									
Day	A†	B†	C†	D†	E†	F†	G†	H†	I†	J†
Dilution Water Control										
1	N	N	N	N	N	N	N	N	N	N
2	0	0	0	0	0	0	0	0	0	0
3	N	N	N	N	N	N	N	N	N	N
4	N	N	N	N	N	N	N	N	N	N
5	0	0	0	0	0	0	0	0	0	0
6	N	N	N	N	N	N	N	N	N	N
7	0	0	0	0	0	0	0	0	0	0
8	N	N	N	N	N	N	N	N	N	N
9	14	16	0	15	27	24	24	25	18	22
10	N	N	Y	N	N	N	N	N	N	N
11	N	N	Y	N	N	N	N	N	N	N
12	44	46	57	48	47	46	50	48	49	47
13	N	N	Y	N	N	N	N	N	N	N
14	0	0	5	0	0	0	0	0	0	0
15	N	Y	N	Y	Y	Y	Y	Y	Y	Y
16	28	37	53	48	51	54	46	49	45	32
17	N	N	N	N	N	N	N	N	N	N
18	N	N	N	N	N	N	N	N	Y	N
19	42	27	44	23	12	47	42	28	32	25
20	N	N	N	N	N	N	N	N	N	N
21	0	0	0	0	0	0	0	0	0	0

† One daphnid per replicate chamber at test start
Y neonates produced but not counted (for non-renewal days only)
N no neonates produced (for non-renewal days only)
0 no neonates produced (for renewal days only)
- not applicable due to adult immobility

Table 10
Number of Live Young Produced by *Daphnia magna* Exposed to H-28548 for 21 Days
(Continued)

	Number of Live Young Produced									
	Replicate									
Day	A†	B†	C†	D†	E†	F†	G†	H†	I†	J†
2.13 mg/L										
1	N	N	N	N	N	N	N	N	N	N
2	0	0	0	0	0	0	0	0	0	0
3	N	N	N	N	N	N	N	N	N	N
4	N	N	N	N	N	N	N	N	N	N
5	0	0	0	0	0	0	0	0	0	0
6	N	N	N	N	N	N	N	N	N	N
7	0	0	0	0	0	0	0	0	0	0
8	N	N	N	N	N	N	N	N	N	N
9	0	0	24	15	29	14	30	0	0	16
10	Y	Y	N	N	N	N	N	Y	N	N
11	Y	Y	N	N	N	N	N	Y	Y	N
12	65	58	50	41	44	33	40	65	16	49
13	N	N	N	N	N	N	N	N	N	N
14	0	0	0	0	0	0	0	0	50	0
15	N	N	N	N	Y	Y	Y	N	N	Y
16	47	49	25	30	32	24	42	47	0	39
17	N	N	N	N	N	N	N	N	Y	N
18	N	N	N	N	N	N	N	N	Y	N
19	27	26	40	46	34	8	43	18	40	21
20	N	N	N	N	N	N	N	N	N	N
21	0	0	0	0	0	0	0	0	47	0

† One daphnid per replicate chamber at test start
Y neonates produced but not counted (for non-renewal days only)
N no neonates produced (for non-renewal days only)
0 no neonates produced (for renewal days only)
- not applicable due to adult immobility

Table 10
Number of Live Young Produced by *Daphnia magna* Exposed to H-28548 for 21 Days
(Continued)

	Number of Live Young Produced									
	Replicate									
Day	A†	B†	C†	D†	E†	F†	G†	H†	I†	J†
4.17 mg/L										
1	N	N	N	N	N	N	N	N	N	N
2	0	0	0	0	0	0	0	0	0	0
3	N	N	N	N	N	N	N	N	N	N
4	N	N	N	N	N	N	N	N	N	N
5	0	0	0	0	0	0	0	0	0	0
6	N	N	N	N	N	N	N	N	N	N
7	0	0	0	0	0	0	0	0	0	0
8	N	N	N	N	N	N	N	N	N	N
9	6	20	22	23	20	19	11	23	0	11
10	N	N	N	N	N	N	N	N	N	N
11	N	Y	N	N	N	Y	N	N	Y	Y
12	38	53	41	47	50	28	33	48	24	33
13	N	N	N	N	N	N	N	N	N	Y
14	0	0	0	0	0	0	0	0	48	46
15	Y	Y	Y	Y	Y	Y	Y	Y	N	N
16	38	41	34	47	51	41	45	44	0	0
17	N	N	N	N	N	N	N	N	Y	Y
18	Y	N	N	N	Y	N	N	N	Y	Y
19	48	16	0	33	11	41	41	36	26	49
20	N	N	N	N	N	N	N	N	Y	Y
21	0	0	0	0	0	0	0	0	42	43

† One daphnid per replicate chamber at test start
Y neonates produced but not counted (for non-renewal days only)
N no neonates produced (for non-renewal days only)
0 no neonates produced (for renewal days only)
- not applicable due to adult immobility

Table 10
Number of Live Young Produced by *Daphnia magna* Exposed to H-28548 for 21 Days
(Continued)

	Number of Live Young Produced									
	Replicate									
Day	A†	B†	C†	D†	E†	F†	G†	H†	I†	J†
8.13 mg/L										
1	N	N	N	N	N	N	N	N	N	N
2	0	0	0	0	0	0	0	0	0	0
3	N	N	N	N	N	N	N	N	N	N
4	N	N	N	N	N	N	N	N	N	N
5	0	0	0	0	0	0	0	0	0	0
6	N	N	N	N	N	N	N	N	N	N
7	0	0	0	0	0	0	0	0	0	0
8	N	N	N	N	N	N	N	N	N	N
9	10	26	12	23	27	31	13	17	14	17
10	N	N	N	N	N	N	N	N	N	N
11	N	N	N	N	N	N	N	N	N	N
12	50	44	49	39	51	42	49	49	38	51
13	N	N	N	N	N	N	N	N	N	N
14	0	0	0	0	0	0	0	0	0	0
15	Y	Y	Y	Y	Y	Y	-	Y	Y	Y
16	34	38	42	33	32	39	-	38	36	36
17	N	N	N	N	N	N	-	N	N	N
18	N	N	Y	N	N	N	-	N	N	N
19	28	23	48	17	29	19	-	22	14	22
20	N	N	N	N	N	N	-	N	N	N
21	0	0	0	0	0	0	-	0	0	0

† One daphnid per replicate chamber at test start
Y neonates produced but not counted (for non-renewal days only)
N no neonates produced (for non-renewal days only)
0 no neonates produced (for renewal days only)
- not applicable due to adult immobility

Table 10
Number of Live Young Produced by *Daphnia magna* Exposed to H-28548 for 21 Days
(Continued)

	Number of Live Young Produced									
	Replicate									
Day	A†	B†	C†	D†	E†	F†	G†	H†	I†	J†
16.2 mg/L										
1	N	N	N	N	N	N	N	N	N	N
2	0	0	0	0	0	0	0	0	0	0
3	N	N	N	N	N	N	N	N	N	N
4	N	N	N	N	N	N	N	N	N	N
5	0	0	0	0	0	0	0	0	0	0
6	N	N	N	N	N	N	N	N	N	N
7	0	0	0	0	0	0	0	0	0	0
8	N	N	N	N	N	N	N	N	N	N
9	0	26	5	23	0	10	20	17	23	17
10	Y	N	Y	N	Y	Y	N	N	N	N
11	Y	N	Y	N	Y	Y	N	Y	N	N
12	52	39	56	45	64	53	35	49	48	41
13	N	N	N	N	N	N	N	N	N	N
14	0	0	0	0	0	0	0	0	0	0
15	N	N	N	N	N	N	Y	Y	Y	Y
16	50	26	46	39	40	40	39	46	39	34
17	N	N	N	N	N	N	N	N	N	N
18	N	N	N	N	N	N	N	Y	N	Y
19	48	31	33	12	37	41	0	8	0	9
20	N	N	N	N	N	N	N	N	N	N
21	0	0	0	0	0	0	0	0	0	0

† One daphnid per replicate chamber at test start
Y neonates produced but not counted (for non-renewal days only)
N no neonates produced (for non-renewal days only)
0 no neonates produced (for renewal days only)
- not applicable due to adult immobility

Table 10
Number of Live Young Produced by *Daphnia magna* Exposed to H-28548 for 21 Days
(Continued)

	Number of Live Young Produced									
	Replicate									
Day	A†	B†	C†	D†	E†	F†	G†	H†	I†	J†
33.0 mg/L										
1	N	N	N	N	N	N	N	N	N	N
2	0	0	0	0	0	0	0	0	0	0
3	N	N	N	N	N	N	N	N	N	N
4	N	N	N	N	N	N	N	N	N	N
5	0	0	0	0	0	0	0	0	0	0
6	N	N	N	N	N	N	N	N	N	N
7	0	0	0	0	0	0	0	0	0	0
8	N	N	N	N	N	N	N	N	N	N
9	10	15	27	0	0	24	28	12	22	0
10	N	N	N	N	N	N	N	N	N	N
11	N	N	N	Y	Y	N	N	N	N	Y
12	50	34	49	24	12	42	47	44	44	13
13	N	N	N	N	N	N	N	N	N	N
14	0	0	0	46	40	0	0	0	0	31
15	Y	Y	Y	N	N	Y	Y	Y	Y	N
16	46	25	38	0	0	25	34	26	41	0
17	N	N	N	Y	Y	N	N	N	N	Y
18	N	Y	N	Y	Y	N	N	Y	N	Y
19	28	36	26	31	47	29	0	0	23	22
20	N	N	N	Y	Y	N	N	N	N	Y
21	0	0	0	48	47	0	0	0	0	39

† One daphnid per replicate chamber at test start
Y neonates produced but not counted (for non-renewal days only)
N no neonates produced (for non-renewal days only)
0 no neonates produced (for renewal days only)
- not applicable due to adult immobility

Table 11
Number of Immobile Young Produced by *Daphnia magna* Exposed to H-28548 for 21 Days

	Number of Immobile Young Produced									
	Replicate									
Day	A†	B†	C†	D†	E†	F†	G†	H†	I†	J†
Dilution Water Control										
1	N	N	N	N	N	N	N	N	N	N
2	0	0	0	0	0	0	0	0	0	0
3	N	N	N	N	N	N	N	N	N	N
4	N	N	N	N	N	N	N	N	N	N
5	0	0	0	0	0	0	0	0	0	0
6	N	N	N	N	N	N	N	N	N	N
7	0	0	0	0	0	0	0	0	0	0
8	N	N	N	N	N	N	N	N	N	N
9	0	0	0	0	0	0	0	0	0	0
10	N	N	N	N	N	N	N	N	N	N
11	N	N	N	N	N	N	N	N	N	N
12	0	0	0	0	0	0	0	0	0	0
13	N	N	N	N	N	N	N	N	N	N
14	0	0	0	0	0	0	0	0	0	0
15	N	N	N	N	N	N	N	N	N	N
16	0	0	0	0	0	0	0	0	0	0
17	N	N	N	N	N	N	N	N	N	N
18	N	N	N	N	N	N	N	N	N	N
19	0	0	0	0	0	0	0	0	0	0
20	N	N	N	N	N	N	N	N	N	N
21	0	0	0	0	0	0	0	0	0	0

† One daphnid per replicate chamber at test start
Y neonates produced but not counted (for non-renewal days only)
N no neonates produced (for non-renewal days only)
0 no neonates produced (for renewal days only)
- not applicable due to adult immobility

Table 11
Number of Immobile Young Produced by *Daphnia magna* Exposed to H-28548 for 21 Days
(Continued)

	Number of Immobile Young Produced									
	Replicate									
Day	A†	B†	C†	D†	E†	F†	G†	H†	I†	J†
2.13 mg/L										
1	N	N	N	N	N	N	N	N	N	N
2	0	0	0	0	0	0	0	0	0	0
3	N	N	N	N	N	N	N	N	N	N
4	N	N	N	N	N	N	N	N	N	N
5	0	0	0	0	0	0	0	0	0	0
6	N	N	N	N	N	N	N	N	N	N
7	0	0	0	0	0	0	0	0	0	0
8	N	N	N	N	N	N	N	N	N	N
9	0	0	0	0	0	0	0	0	0	0
10	N	N	N	N	N	N	N	N	N	N
11	N	N	N	N	N	N	N	N	N	N
12	0	0	0	0	0	0	0	0	0	3
13	N	N	N	N	N	N	N	N	N	N
14	0	0	0	0	0	0	0	0	0	0
15	N	N	N	N	N	N	N	N	N	N
16	0	0	0	0	0	0	0	0	0	0
17	N	N	N	N	N	N	N	N	N	N
18	N	N	N	N	N	N	N	N	N	N
19	0	0	0	0	0	0	0	20	0	0
20	N	N	N	N	N	N	N	N	N	N
21	0	0	0	0	0	0	0	0	0	0

† One daphnid per replicate chamber at test start
Y neonates produced but not counted (for non-renewal days only)
N no neonates produced (for non-renewal days only)
0 no neonates produced (for renewal days only)
- not applicable due to adult immobility

Table 11
Number of Immobile Young Produced by *Daphnia magna* Exposed to H-28548 for 21 Days
(Continued)

	Number of Immobile Young Produced									
	Replicate									
Day	A†	B†	C†	D†	E†	F†	G†	H†	I†	J†
4.17 mg/L										
1	N	N	N	N	N	N	N	N	N	N
2	0	0	0	0	0	0	0	0	0	0
3	N	N	N	N	N	N	N	N	N	N
4	N	N	N	N	N	N	N	N	N	N
5	0	0	0	0	0	0	0	0	0	0
6	N	N	N	N	N	N	N	N	N	N
7	0	0	0	0	0	0	0	0	0	0
8	N	N	N	N	N	N	N	N	N	N
9	0	1	0	0	0	0	0	0	0	0
10	N	N	N	N	N	N	N	N	N	N
11	N	N	N	N	N	N	N	N	N	N
12	0	0	0	0	0	0	0	0	0	0
13	N	N	N	N	N	N	N	N	N	N
14	0	0	0	0	0	0	0	0	0	0
15	N	N	N	N	N	N	N	N	N	N
16	0	0	0	0	0	0	0	0	0	0
17	N	N	N	N	N	N	N	N	N	N
18	N	N	N	N	N	N	N	N	N	N
19	0	0	43	0	35	0	0	6	0	0
20	N	N	N	N	N	N	N	N	N	N
21	0	0	0	0	0	0	0	0	0	0

† One daphnid per replicate chamber at test start
Y neonates produced but not counted (for non-renewal days only)
N no neonates produced (for non-renewal days only)
0 no neonates produced (for renewal days only)
- not applicable due to adult immobility

Table 11
Number of Immobile Young Produced by *Daphnia magna* Exposed to H-28548 for 21 Days
(Continued)

	Number of Immobile Young Produced									
	Replicate									
Day	A†	B†	C†	D†	E†	F†	G†	H†	I†	J†
8.13 mg/L										
1	N	N	N	N	N	N	N	N	N	N
2	0	0	0	0	0	0	0	0	0	0
3	N	N	N	N	N	N	N	N	N	N
4	N	N	N	N	N	N	N	N	N	N
5	0	0	0	0	0	0	0	0	0	0
6	N	N	N	N	N	N	N	N	N	N
7	0	0	0	0	0	0	0	0	0	0
8	N	N	N	N	N	N	N	N	N	N
9	10	0	0	0	0	0	3	0	0	0
10	N	N	N	N	N	N	N	N	N	N
11	N	N	N	N	N	N	N	N	N	N
12	0	0	0	0	0	0	0	0	1	0
13	N	N	N	N	N	N	N	N	N	N
14	0	0	0	0	0	0	0	0	0	0
15	N	N	N	N	N	N	-	N	N	N
16	0	0	0	0	0	0	-	0	0	0
17	N	N	N	N	N	N	-	N	N	N
18	N	N	N	N	N	N	-	N	N	N
19	2	2	0	0	0	0	-	0	8	0
20	N	N	N	N	N	N	-	N	N	N
21	0	0	0	0	0	0	-	0	0	0

† One daphnid per replicate chamber at test start
Y neonates produced but not counted (for non-renewal days only)
N no neonates produced (for non-renewal days only)
0 no neonates produced (for renewal days only)
- not applicable due to adult immobility

Table 11
Number of Immobile Young Produced by *Daphnia magna* Exposed to H-28548 for 21 Days
(Continued)

	Number of Immobile Young Produced									
	Replicate									
Day	A†	B†	C†	D†	E†	F†	G†	H†	I†	J†
16.2 mg/L										
1	N	N	N	N	N	N	N	N	N	N
2	0	0	0	0	0	0	0	0	0	0
3	N	N	N	N	N	N	N	N	N	N
4	N	N	N	N	N	N	N	N	N	N
5	0	0	0	0	0	0	0	0	0	0
6	N	N	N	N	N	N	N	N	N	N
7	0	0	0	0	0	0	0	0	0	0
8	N	N	N	N	N	N	N	N	N	N
9	0	0	0	0	0	0	0	0	0	0
10	N	N	N	N	N	N	N	N	N	N
11	N	N	N	N	N	N	N	N	N	N
12	0	0	0	0	0	0	0	0	0	1
13	N	N	N	N	N	N	N	N	N	N
14	0	0	0	0	0	0	0	0	0	0
15	N	N	N	N	N	N	N	N	N	N
16	0	0	0	0	0	0	0	0	0	0
17	N	N	N	N	N	N	N	N	N	N
18	N	N	N	N	N	N	N	N	N	N
19	3	2	0	0	0	0	28	20	36	29
20	N	N	N	N	N	N	N	N	N	N
21	0	0	0	0	0	0	0	0	0	0

† One daphnid per replicate chamber at test start
Y neonates produced but not counted (for non-renewal days only)
N no neonates produced (for non-renewal days only)
0 no neonates produced (for renewal days only)
- not applicable due to adult immobility

Table 11
Number of Immobile Young Produced by *Daphnia magna* Exposed to H-28548 for 21 Days
(Continued)

	Number of Immobile Young Produced									
	Replicate									
Day	A†	B†	C†	D†	E†	F†	G†	H†	I†	J†
33.0 mg/L										
1	N	N	N	N	N	N	N	N	N	N
2	0	0	0	0	0	0	0	0	0	0
3	N	N	N	N	N	N	N	N	N	N
4	N	N	N	N	N	N	N	N	N	N
5	0	0	0	0	0	0	0	0	0	0
6	N	N	N	N	N	N	N	N	N	N
7	0	0	0	0	0	0	0	0	0	0
8	N	N	N	N	N	N	N	N	N	N
9	0	0	0	0	0	1	1	4	0	0
10	N	N	N	N	N	N	N	N	N	N
11	N	N	N	N	N	N	N	N	N	N
12	0	0	0	0	0	0	0	0	0	0
13	N	N	N	N	N	N	N	N	N	N
14	0	0	0	0	0	0	0	0	0	0
15	N	N	N	N	N	N	N	N	N	N
16	0	0	0	0	0	0	0	0	0	0
17	N	N	N	N	N	N	N	N	N	N
18	N	N	N	N	N	N	N	N	N	N
19	7	8	9	0	0	0	19	29	0	0
20	N	N	N	N	N	N	N	N	N	N
21	0	0	0	0	0	0	0	0	0	0

† One daphnid per replicate chamber at test start
Y neonates produced but not counted (for non-renewal days only)
N no neonates produced (for non-renewal days only)
0 no neonates produced (for renewal days only)
- not applicable due to adult immobility

Table 12
Reproduction Data at Test Conclusion for *Daphnia magna* Exposed to H-28548 for 21 Days

Mean, Measured H-28548 Concentration (mg/L)	Replicate									
	A†	B†	C†	D†	E†	F†	G†	H†	I†	J†
Dilution Water Control										
1st Reproduction Day[a]	9	9	10	9	9	9	9	9	9	9
Total Live Young[b]	128	126	159	134	137	171	162	150	144	126
Total Immobile Young[c]	0	0	0	0	0	0	0	0	0	0
2.13										
1st Reproduction Day[a]	10	10	9	9	9	9	9	10	11	9
Total Live Young[b]	139	133	139	132	139	79	155	130	153	125
Total Immobile Young[c]	0	0	0	0	0	0	0	20	0	3
4.17										
1st Reproduction Day[a]	9	9	9	9	9	9	9	9	11	9
Total Live Young[b]	130	130	97	150	132	129	130	151	140	182
Total Immobile Young[c]	0	1	43	0	35	0	0	6	0	0

† One daphnid per replicate chamber at test start
X Not applicable due to total adult immobility
a First day of reproduction
b Sum of live young produced per surviving adult at test end
c Sum of immobile young produced per surviving adult at test end

Table 12
Reproduction Data at Test Conclusion for *Daphnia magna* Exposed to H-28548 for 21 Days (Continued)

Mean, Measured H-28548 Concentration (mg/L)	Replicate									
	A†	B†	C†	D†	E†	F†	G†	H†	I†	J†
8.13										
1st Reproduction Day[a]	9	9	9	9	9	9	9	9	9	9
Total Live Young[b]	122	131	151	112	139	131	X	126	102	126
Total Immobile Young[c]	12	2	0	0	0	0	X	0	9	0
16.2										
1st Reproduction Day[a]	10	9	9	9	10	9	9	9	9	9
Total Live Young[b]	150	122	140	119	141	144	94	120	110	101
Total Immobile Young[c]	3	2	0	0	0	0	28	20	36	30
33.0										
1st Reproduction Day[a]	9	9	9	11	11	9	9	9	9	11
Total Live Young[b]	134	110	140	149	146	120	109	82	130	105
Total Immobile Young[c]	7	8	9	0	0	1	20	33	0	0

† One daphnid per replicate chamber at test start
X Not applicable due to total adult immobility
a First day of reproduction
b Sum of live young produced per surviving adult at test end
c Sum of immobile young produced per surviving adult at test end

Table 13
Length in Millimeters at Test Conclusion for *Daphnia magna* Exposed to H-28548 for 21 Days

Mean, Measured H-28548 Concentrations (mg/L)	Length (mm)									
	Replicate									
	A†	B†	C†	D†	E†	F†	G†	H†	I†	J†
Dilution Water Control	4.42	4.03	4.29	4.42	4.29	4.42	4.55	4.16	4.55	4.03
2.13	4.42	4.29	4.81	4.42	4.81	4.81	4.42	4.16	4.55	4.29
4.17	4.68	4.29	4.94	4.42	4.42	4.55	3.9	4.03	4.29	4.68
8.13	4.16	4.29	4.55	4.29	4.29	3.9	X	4.16	4.55	4.29
16.2	4.55	4.42	4.29	4.29	4.03	4.42	4.42	4.68	4.42	4.68
33.0	4.29	4.42	4.68	4.55	4.16	4.55	4.55	4.29	4.29	4.03

† One daphnid per replicate chamber at test start.
X Not applicable due to total adult immobility.

Table 14
Dry Weight in Milligrams at Test Conclusion for *Daphnia magna* Exposed to H-28548 for 21 Days

Mean, Measured H-28548 Concentrations (mg/L)	Dry Weight (mg)†									
	1	2	3	4	5	6	7	8	9	10
Dilution Water										
Control	0.95	0.97	0.96	1.00	0.72	0.64	0.87	1.00	1.00	0.88
2.13	0.34	0.92	0.85	0.91	1.12	0.90	0.56	0.81	0.90	0.80
4.17	0.87	0.76	0.90	0.47	0.74	0.87	0.60	0.57	1.06	0.82
8.13	0.79	1.00	0.81	0.61	0.80	0.84	0.81	0.62	0.66	X
16.2	0.60	0.81	0.71	0.95	0.95	0.84	0.77	0.91	0.90	0.76
33.0	0.82	1.02	0.93	0.38	0.85	0.76	0.33	0.87	0.91	0.99

† Replicate identification was not maintained during weighing.
X Not applicable due to total adult immobility.

Table 15
Summary Table of Statistical Endpoints

ENDPOINT	NOEC (mg/L)	MATC (mg/L)	LOEC (mg/L)	STATISTICAL TEST
Length (mm) @21 Days	>33.0	*	*	Jonckheere-Terpstra
Weight (mg) @21 Days	>33.0	*	*	Jonckheere-Terpstra
Immobility @ 21 Days	>33.0	*	*	Fisher Exact, Cochran-Armitage
First Day Reproduction	>33.0	*	*	Jonckheere-Terpstra
Total Live Young @ 21 Days	4.17	6.15	8.13	Jonckheere-Terpstra
Immobilized Young @ 21 Days	8.13	12.2	16.2	Jonckheere-Terpstra

* Not calculated/could not be adequately determined

FIGURES

Figure 1
Representative Analytical Calibration Standard Curve for H-28548

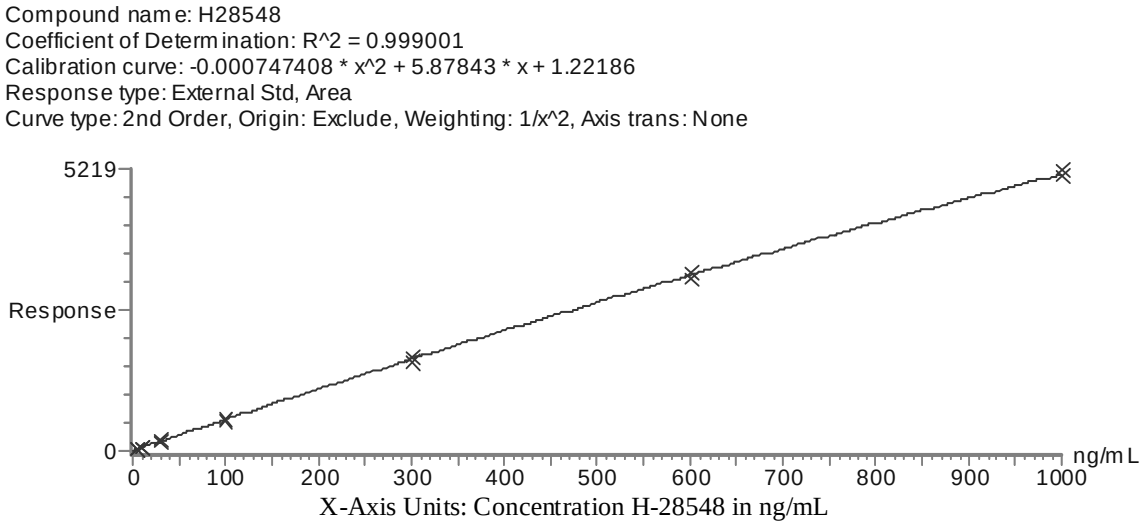
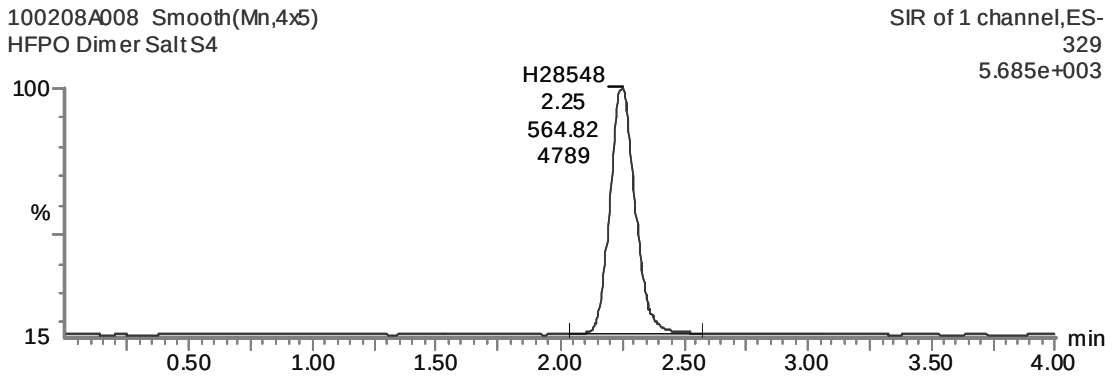
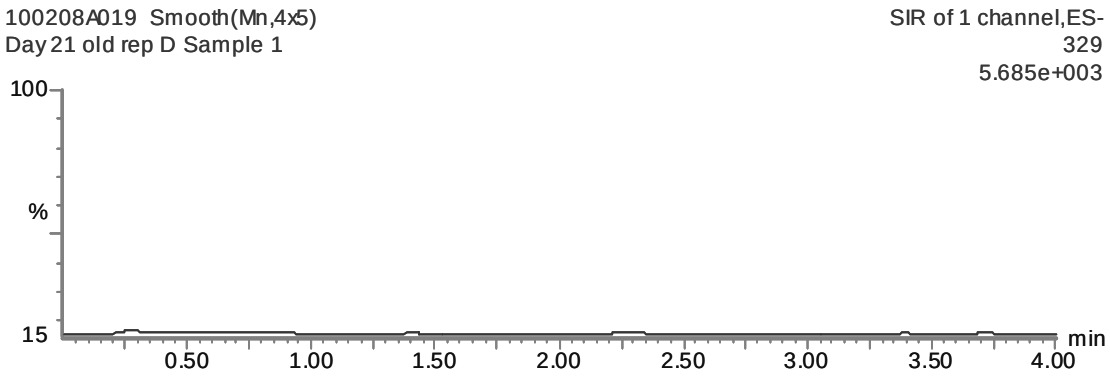


Figure 2
Representative HPLC Chromatogram of a Calibration Standard Solution



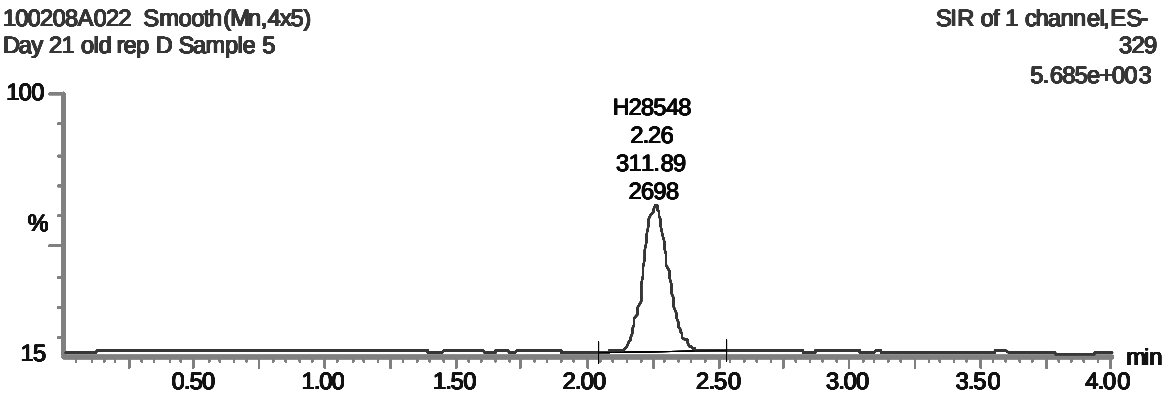
H-28548 elutes at a retention time of approximately 2.1 to 2.3 minutes.
The calibration standard solution contains H-28548 at a concentration of 100 ng/mL.

Figure 3
Representative HPLC Chromatogram of a Dilution Water Control Solution



H-28548 would elute at a retention time of approximately 2.1 to 2.3 minutes.

Figure 4
Representative HPLC Chromatogram of a H-28548 Test Solution



H-28548 elutes at a retention time of approximately 2.1 to 2.3 minutes.
Test solution sample contains H-28548 at a nominal concentration of 2.1 mg/L.

APPENDIX

Appendix A
Certificate of Analysis

E. I. du Pont de Nemours and Company
Wilmington, DE 19898
USA

CERTIFICATE OF ANALYSIS

This Certificate of Analysis fulfills the requirement for characterization of a test substance prior to a study subject to GLP regulations. It documents the identity and content of the test substance. This work was conducted under EPA Good Laboratory Practice Standards (40 CFR 792).

Haskell Code Number	H-28548
Common Name	HFPO Dimer Acid Ammonium Salt
Purity Percent	84%
Other Components	Water – 12.7% Perfluorooctanoic acid – 150 ppm
Date of Analysis	June 13, 2008
Recommended reanalysis interval	1 year
Instructions for storage	NRT&H
Reference	DuPont-25455
Analysis performed at	E. I. DuPont de Nemours and Company DuPont Haskell Laboratories Newark, Delaware USA

Approver:

Peter A. Bloxham, Ph.D.
Senior Research Chemist

18-JUNE-2008
Date